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Trimethyltin-induced alterations in behavior are linked to changes in PSA-NCAM expression

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Abstract

The neurotoxic heavy metal trimethyltin (TMT) primarily damages neurons of the hippocampus and limbic areas of the temporal lobe, and causes a dose-dependent decrease in the polysialated form of the neural cell adhesion molecule (PSA-NCAM) in the mouse hippocampus. In the current study, we attempted to associate deficits in spatial learning following TMT exposure at various stages in learning with changes in levels of NCAM-180 and PSA-NCAM in both the hippocampus and frontal cortex. Mice were treated with TMT either before or after training on a spatial learning paradigm and examined for changes in NCAM and PSA-NCAM 12 h later. In the first set of experiments, male BALB/c mice were injected with TMT (2.25 mg/kg) or saline i.p. and tested 24–168 h later using hidden and visible versions of the water maze, as well as light avoidance and motor activity. Mice in both treated and control groups which demonstrated a significant improvement in water maze performance also showed an elevation in hippocampal PSA-NCAM at all time points examined. TMT exposure impaired spatial learning and blocked learning-induced elevations in PSA-NCAM expression 24–96 h post-treatment, but these deficits disappeared by 168 h post-treatment. Mice exposed to TMT during reconsolidation of spatial learning (after repeated water maze training) demonstrated a mild and transient difference in escape latency compared to saline exposed mice. TMT administration during this period did not result in the attenuation of PSA-NCAM expression observed when animals were exposed before training. These results confirm a specific role for PSA-NCAM in acquisition and consolidation of spatial memory.

Keywords: Neurotoxicity; PSA-NCAM; Mouse; Spatial learning; Hippocampus

1. Introduction

Trimethyltin (TMT) is a potent neurotoxicant which produces a dose- and species-dependent degeneration of neurons in the limbic system (Chang et al., 1983; Dyer et al., 1982; Earley et al., 1992), particularly the hippocampus, amygdala and entorhinal cortex (Balaban et al., 1988; Dyer et al., 1982; McMillan and Wenger, 1985). Damage to the hippocampal formation following TMT treatment in rats includes a loss of pyramidal and granule cell neurons (Dyer et al., 1982; Fiedorowicz et al., 2001; Valdes et al., 1983; for review see McMillan and Wenger, 1985), neuronal necrosis (Bouldin et al., 1981; Chang et al., 1982; Geloso et al., 1998; Valdes et al., 1983), apoptosis (Fiedorowicz et al., 2001) and increased expression of GFAP (McCann et al., 1996; Fiedorowicz et al., 2001; Haga et al., 2002). In the rat, TMT impairs hippocampally-dependent behaviors, including passive avoidance (Walsh et al., 1982a), water maze (Earley et al., 1992; Hagan et al., 1988) and working and/or reference memory measured in the radial arm maze (Alessandri et al., 1994; Bushnell and Angell, 1992; Miller and O'Callaghan, 1984; Walsh et al., 1982b). Concomitantly, TMT administration also results in a long-lasting hyperactivity (Hagan et al., 1988; Johnson et al., 1984; Miller and O'Callaghan, 1984; Swartzwelder et al., 1982), consistent with reports of increased motor activity following lesion-induced hippocampal damage (Coutureau et al., 2000; Galani et al., 1998; Good and Honey, 1997; Moses et al., 2002).

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While much of the pathological and behavioral characterization of TMT has been performed in rats, TMT also induces a characteristic spectrum of neurotoxicity in mice (Chang et al., 1982). Mice are more susceptible to damage of the hippocampal granule cell layer than are rats, displaying neuronal damage at lower doses and at much earlier time points (Chang et al., 1983). Despite behavioral consequences of doses as low as 2.0 mg/kg (Wenger et al., 1984a) significant morphological changes are only observed following doses $\geq$3.0 mg/kg in the BALB/c mouse, at which hyperactivity and altered fixed-interval responding are seen (Chang et al., 1983; Dyer et al., 1982; Wenger et al., 1984a,b). This indicates that behavioral impairments at doses below 3.0 mg/kg in the BALB/c mouse are not solely the result of hippocampal granule cell necrosis.

Administration of TMT at doses between 2.0 and 2.75 mg/kg results in a loss of the 180 kDa isoform of the neural cell adhesion molecule (NCAM-180) (Dey et al., 1994) and of the polysialated form of NCAM (PSA-NCAM) (Dey et al., 1997). NCAM is a morphoregulatory molecule developmentally expressed on the neuronal membrane. During development, NCAM plays a major role in neuron–neuron adhesion and neuron–axon adhesion. During neurogenesis, migration and neurite formation, neurons express an isoform of NCAM containing chains of polysialic acid residues on its extracellular domain (Edelman, 1984; Rutishauser et al., 1988; Rutishauser and Landmesser, 1996). These negatively-charged sialic acid chains serve to inhibit NCAM-mediated adhesion during migration, allowing neurons to respond to extracellular signals (e.g. growth factors) and preventing them from inappropriately adhering to each other during neural morphogenesis (Bruses and Rutishauser, 2001; Cremer et al., 2000; Fujimoto et al., 2001; Kiss et al., 2001; Rutishauser et al., 1988; Rutishauser and Landmesser, 1996; Seki and Arai, 1993). In addition to its developmental functions, a role for PSA-NCAM has been demonstrated in both the maintenance of stable synapses and synaptic plasticity during learning (Becker et al., 1996; DeStephano et al., 2001; Doyle et al., 1992; Kiss et al., 2001).

While PSA-NCAM expression is down-regulated in most brain areas during neuronal maturation, PSA-NCAM continues to be highly expressed in areas of the adult brain that undergo cellular and molecular plasticity, especially the hippocampus (Ni Dhull et al., 1999; Seki and Arai, 1993). Increased PSA-NCAM expression is observed in the hippocampus and pyriform cortex during periods of consolidation of passive avoidance and water maze training (Becker et al., 1996; Doyle and Regan, 1993; Fox et al., 2000; Murphy and Regan, 1999). Modulation of PSA-NCAM expression is thought to underlie synaptic remodeling events which facilitate memory storage and stabilization following initial training, termed consolidation (Bruses and Rutishauser, 2001; Cremer et al., 2000; Fox et al., 2000; Kiss et al., 2001). This period can be differentiated from reconsolidation, in which repeated retrieval and reactivation of the previously consolidated memory during new experiences either stabilizes or reorganizes previously learned information (Sara, 2000; Walker et al., 2003).

Reduction in PSA-NCAM expression during senescence or enzymatic removal of PSA from the neuronal membrane with endoneuraminidase is associated with impaired performance in the water maze (Cremer et al., 2000; Doyle et al., 1992), and improvements in hippocampal-mediated contextual fear conditioning and spatial learning are observed following activation of the NCAM-binding site of the FGF1 receptor (FGFR1) (Cambon et al., 2004). Synaptogenesis consequent to activation of this receptor is mediated through PSA-NCAM (Dityatev et al., 2004), indicating that the PSA-NCAM-FGFR1 interaction may serve as a central mechanism of learning-induced synaptogenesis.

The present study examined the relationship between TMT-induced deficits of a spatial learning task and changes in PSA-NCAM expression in the mouse when TMT was administered during acquisition and reconsolidation. A dose of TMT was selected that reduced the level of hippocampal PSA-NCAM in the mouse without causing appreciable neuronal death (Dey et al., 1997). The reversible nature of TMT-induced reduction in hippocampal PSA-NCAM levels (Dey et al., 1997) was exploited to determine if the level of PSA-NCAM expression related to impairment of behavioral performance. These results were compared with expression levels in the frontal cortex, an area involved in consolidation and retention of spatial learning (Maviel et al., 2004; Vafaei and Rashidy-Pour, 2004).

In addition to spatial navigation using the water maze, TMT-treated mice were evaluated on light avoidance and motor activity paradigms, thus providing additional data on TMT-induced disruption of PSA-NCAM expression on motor and anxiety mediated behaviors. These tasks were chosen because previous studies have reported alterations in the performance of NCAM knockout mice tested in these paradigms (Stork et al., 1999, 2000). Homozygous and heterozygous NCAM knockout mice spend more time in a darkened side of light avoidance task along with increased exploration and rearing in this paradigm. To examine whether TMT exposure produced similar deficits as a consequence of loss of PSA-NCAM, animals were treated with either TMT or saline and tested on the light avoidance task at time points which corresponded to reduction and recovery of PSA-NCAM levels following TMT (24–168 h post treatment). These results support the requirement for proper PSA-NCAM expression during acquisition and consolidation of memory.

2. Materials and methods

2.1. Animals

Male BALB/c mice aged 6–9 weeks (Taconic, NY) were housed in pairs in shoebox type cages with wood chip bedding according to AALAC guidelines. Twenty-four hours prior to water maze training, mice were habituated to the task by allowing them to swim freely in the maze for 10, 1-min periods. To determine the time course of TMT-induced behavioral impairments and changes in PSA-NCAM expression, mice were treated with either 2.25 mg/kg trimethyltin chloride i.p. (Aldrich, Milwaukee, WI) or an equal volume of saline following habituation to the water maze. Animals were

maintained in their home cage for 24, 96 or 168 h, then trained in the water maze paradigm (details provided below). In order to prevent behavioral changes as a consequence of multiple behavioral training trials per day, an additional group of animals was tested 24–168 h in the light avoidance and motor activity paradigms. The last group of mice was trained for three consecutive days, treated with a single i.p. injection of 2.25 mg/kg TMT or saline 12 h after the 24th training trial (a time period corresponding to reconsolidation), allowed to recover and retested 96 h after injection. To control for potential alterations in expression of PSA-NCAM and NCAM-180 due to swimming stress or alterations in activity during the water maze training task, an additional group of mice was allowed to swim in the water maze without a platform present (''control, no WM''). All mice were sacrificed 12 h after the last trial and frontal cortices and hippocampi were removed on ice and stored at −80 °C.

2.2. Behavioral assessments

2.2.1. Water maze

The water maze consisted of a circular galvanized steel tub (128 cm in diameter and 39 cm deep) painted white and kept in a sound-attenuated room. It was partially filled with tap water made opaque with non-toxic, white latex paint. The temperature of the water was maintained at 24–26 °C. At the beginning of each trial, mice were placed facing the outside of the maze at one of four equi-distant starting positions in a random order. Latency to escape onto a hidden platform (6 cm in diameter, hidden approximately 2 cm below the surface of the water) and time spent swimming in the quadrant holding the hidden platform were recorded. A maximum of 60 s was allowed to find the platform. At the end of each trial, mice remained on the platform and were allowed to rest for 30 s. If the mouse did not find the platform on its own, the experimenter gently led the mouse to the platform. Mice were then removed from the maze, hand-dried, and warmed under a 75 W light bulb during the 15 min intertrial interval. Each mouse was tested for a total of eight trials per day. As a control procedure, some mice were allowed to swim inside the maze with no escape platform for eight trials, 60 s each, with a 15 min intertrial interval. To assess swimming ability, another group of animals was allowed to swim to an identical escape platform, painted black and raised 3 cm above the surface of the water. Each mouse was allowed five trials with a 30 s intertrial interval in the visible platform task.

2.2.2. Light avoidance

Light avoidance was assessed in a three-compartment plexiglass chamber with wire-mesh flooring. The chamber consisted of two sides measuring 28 cm × 34 cm × 32 cm separated by a center compartment (10 cm wide). On one side, a 150-W bulb illuminated white plexiglass walls, while the other compartment was enclosed by black plexiglass and kept covered. The center compartment was constructed of clear plexiglass towards the illuminated side and black plexiglass toward the darkened side. With the exception of the light illuminating the white side of the chamber, the room was dark. Mice were placed in the center compartment facing away from both the light and dark compartments. The door to each compartment was opened and the animal was allowed free access to either side for a period of 5 min. Latency to enter the dark compartment, time spent in each of the two sides, and number of crossings between the light and dark sides were recorded. The box was wiped down with 70% ethanol after each animal.

2.2.3. Horizontal motor activity

Immediately following the 5 min testing period in the light avoidance chamber, each animal was transferred to a locomotor activity box (Columbus Instruments, OH) in a dimly lit room and tested for total horizontal motor activity for 10 min. Motor activity (beam crossing) was detected and recorded by six equally-spaced infrared sensors on each side of a 41 cm × 41 cm plexiglass box.

2.2.4. Statistical analysis of behavioral paradigms

Analysis of escape latency following TMT exposure at each time period was made using a two-way ANOVA with trial and treatment condition as independent variables. Latency to enter the dark compartment, time spent in the lighted side, number of crossings and horizontal motor activity were analyzed using a *t*-test.

2.3. Gel electorphoresis and western blotting

Hippocampi and frontal cortices were dissected and homogenized in 1:10 w/v of a Tris extraction buffer (50 mM Tris–HCl, pH 7.4, 0.32 M sucrose, 1 mM EDTA, 1 vial of protease inhibitor (Sigma, St. Louis, MO) in 100 ml distilled deionized water). Samples were centrifuged for 10 min, the aqueous phase removed, and the pellet combined with equilibration buffer (0.125 M Tris–HCl, pH 6.8, 4% SDS, 20% glycerol, 10% mercaptoethanol), and immediately heated for 30 min at 70 °C. Protein concentrations were determined using the BCA protein assay (Pierce, Rockford, IL) modified for a COBAS FARA II enzyme analyzer (Roche Diagnostics, Nutley, NJ). Samples of 10 μg protein were separated by SDS-PAGE on a 7.5% polyacrylamide gel using a Bio-Rad Mini-Protean II System (Bio-Rad, Mellville, NY). Proteins were transferred to nitrocellulose membranes according to Towbin et al. (1979). Nitrocellulose blots were washed twice for 10 min each in PBS with 0.2% Tween (PBS-T) (Sigma, St. Louis, MO) and blocked with 5% non-fat dry milk in PBS-T for 1 h prior to application of primary antibody. Incubation with antibodies for NCAM and PSA-NCAM was performed overnight at 4 °C. The antibody to total 180 and 140 kDa NCAM (OB-11) was obtained from Sigma (St. Louis, MO) while the antibody for PSA-NCAM (5A5) was a generous gift of Dr. U. Rutishauser. Blots were then incubated with secondary HRP-linked antibodies (Southern Biotechnology Associates, Birmingham, AL) for 1 h and visualized on autoradiographic film using electrochemoluminescence (Amersham, Piscataway, NJ). Films were scanned using a transparency adapter and analyzed for density with ImagePro® analysis software. When samples from the same experiment or time point were run on different gels, density was converted to a

Table 1
Summary of TMT-induced changes in water maze performance and NCAM levels

TMT exposure	Assay point	Water maze performance	Cortical PSA-NCAM	Hippocampal PSA-NCAM
Administered prior to training *(acquisition)*	24 h	*Impairment*	↓	↓
	96 h 108 h (+12h WM)	*Impairment*	Trained in WM: *No change*	↓
			Not trained: ↑	↓
	168 h	*No effect of TMT*	*No change*	*No change*
Administered following acquisition *(during reconsolidation)*	96 h water maze 108 h NCAM	*Transient impairment seen in first few trials*	*No change*	↑

percent of saline-untrained control values. Statistical analysis was performed by ANOVA or *t*-test with treatment condition serving as independent variable and density expressed as a percent of saline-no water maze controls as dependent variable.

3. Results

3.1. Water maze

Table 1 summarizes the primary time-course effects of TMT administration on water maze performance and PSA-NCAM levels. At 24 h post-injection, there was no significant difference between saline and TMT-treated mice on latency to find a visible platform (Fig. 1b). However, the TMT-treated mice were unable to locate a hidden platform ($F(1,10) = 11.33$, $p = 0.01$). While saline-treated animals showed a significant improvement across the eight trials ($F(7,95) = 2.3$, $p = 0.04$), TMT-treated mice swam for the full 60 s without successful escape (Fig. 1a). At 96 h post-injection, saline-treated animals again showed significant improvement in performance, whereas TMT treated mice did not ($F(7,138) = 2.34$, $p = 0.01$) (Fig. 2a)). Again, there were no differences between treatment groups in escape latency when the platform was visible (Fig. 2b). The saline treated animals showed shorter escape latencies and spent a greater proportion of time in the platform quadrant than TMT-treated mice ($F(1,18) = 6.3$, $p = 0.01$) and ($F(1,18) = 5.9$, $p = 0.01$). In contrast, by 168 h post-injection, both groups showed significant improvement by trial 8 ($F(7,52) = 3.6$, $p = 0.003$) with no significant difference between treatment conditions (Fig. 3). When administered during reconsolidation (after acquisition of the water maze task), TMT produced a transient impairment in performance ($F(10,138) = 2.8$, $p < 0.0001$), an effect that was only seen in the first three trials following treatment (Fig. 4).

3.2. Light avoidance and motor activity

Twenty-four hours post-injection, TMT-treated mice demonstrated an anxiogenic response, spending almost no time in the light compartment of the box ($t(11) = 3.11$,

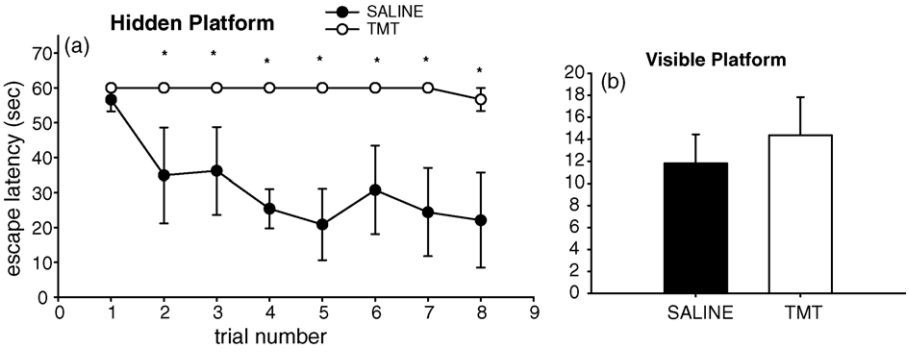

Fig. 1. (a) Impairment in spatial learning 24 h following TMT in the BALB/c mouse; (b) no effect of TMT when the platform was visible and extramaze cues were hidden $n = 6$ per group. (*) Indicates significantly different from saline treated mice using Fisher's PLSD, $p < 0.05$.

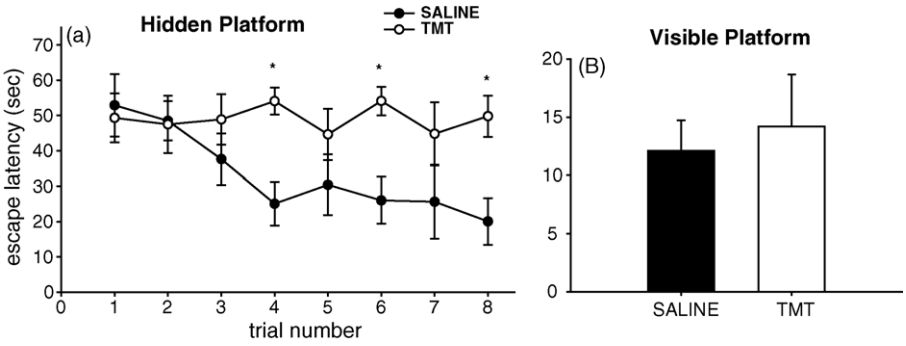

Fig. 2. (a) Impairment in spatial learning following TMT in the BALB/c mouse when tested 96 h after treatment. $n = 12$ TMT treated, $n = 8$ saline treated mice; (b) No effect of TMT when the platform was visible and extramaze cues were hidden. $n = 6$ per group. (*) Indicates significantly different from saline treated mice using Fisher's PLSD, $p < 0.05$.

$p = 0.009$) (Fig. 5). These mice also made fewer transitions from the light to the dark compartment ($t(12) = 3.78$, $p = 0.002$), suggesting that once the subject reached the dark side, they preferred to stay there for the full 5 min testing period. The differences in time spent in the lighted side and reduction in motor activity remained at 96 h post-TMT (Halladay et al., 2003). One week (168 h) after TMT treatment, however, there were no significant differences between the two groups on horizontal motor activity, light/dark transitions, latency to enter the dark compartment or time spent in the light compartment (Fig. 5).

3.3. NCAM and PSA-NCAM Expression

3.3.1. Prior to water maze training

Twenty-four hours after administration, TMT caused a significant loss of PSA-NCAM in both the hippocampus ($t(4) = 6.3$, $p = 0.003$) and the frontal cortex ($t(4) = 3.2$, $p = 0.03$) (Fig. 6). NCAM-180 expression was not altered by TMT treatment (data not shown). At 96 h post-TMT, mice were exposed to water maze training or control paradigms and sacrificed 12 h post-training (108 h post-injection). Analysis revealed a learning-induced increase in PSA-NCAM in the hippocampus, consistent with previous reports (Becker et al., 1996; Murphy and Regan, 1999). At this time point, TMT resulted in an increase in PSA-NCAM in the frontal cortex ($F(3,32) = 12.26$, $p < 0.001$) and a decrease of PSA-NCAM in the hippocampus ($F(3,32) = 12.66$, $<.0009$) in untrained animals, with no changes in total NCAM-180 levels (Fig. 8). In addition to reducing expression in naïve animals, TMT significantly attenuated the water-maze dependent increase in PSA expression in the hippocampus following training (Fig. 7). At 180 h (168 h post TMT, 12 h post water maze) post treatment, PSA-NCAM levels were elevated in mice trained on the water maze, however this increase in PSA-NCAM did not reach significance ($F(2,9) = 5.14$, $p = 0.06$) (Fig. 3). There were no significant differences in NCAM-180 levels when animals were assayed 180 h following TMT exposure (Fig. 8).

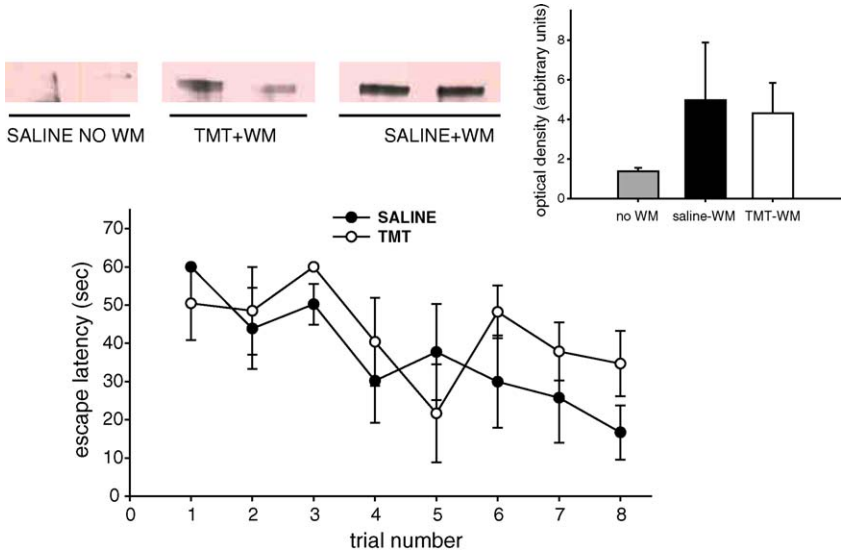

Fig. 3. Top: PSA-NCAM levels in the hippocampus following TMT and saline treatment. Mice were sacrificed 180 h after injection (12 h after water maze training, which occurred 168 h after treatment). $n = 2$ saline no WM, $n = 4$ each saline + WM and TMT + WM. Bottom: escape latency in the water maze when animals were tested 168 h after TMT exposure. $n = 4$ saline and $n = 4$ TMT.

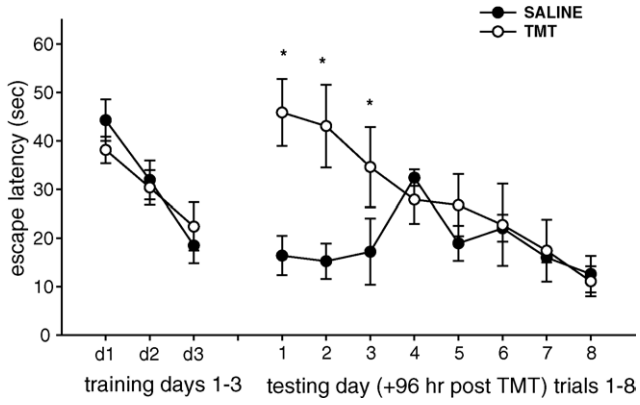

Fig. 4. Effect of TMT or saline treatment when administered 12 h after three consecutive days of water maze training. Testing resumed 96 h after the TMT or saline administration. $n = 10$ TMT and $n = 8$ saline treated mice. (*) Indicates significantly different from saline treated controls using Fisher's PLSD, $p < 0.05$.

3.3.2. TMT administration during reconsolidation

TMT administration did not produce a persistent deficit in spatial navigation when administered following 3 days of training, and both saline and TMT mice demonstrated an elevation in PSA-NCAM compared to saline-no water maze controls ($F(2,24) = 17.5$, $p < 0.0001$) (Fig. 9). Those animals exposed to TMT following 3 days of spatial learning training showed a significantly higher induction of PSA-NCAM following testing on the water maze task compared to saline treated mice. In saline-treated mice trained on the water maze, the increase in PSA-NCAM in the frontal cortex following water maze training approached significance ($p = 0.08$).

4. Discussion

4.1. Time course

The increase in PSA-NCAM during specific periods of memory consolidation (12–24 h after a single learning trial) is thought to underlie synaptic plasticity necessary for formation of new neural circuits during discrete periods of learning (Becker et al., 1996; Doyle and Regan, 1993; Doyle et al., 1992; Murphy et al., 1996). The observation that selective reduction of PSA-NCAM in neurons correlates with a functional impairment in spatial learning is consistent with other studies in which PSA-NCAM was removed by treatment with endoneuraminidase NE (Becker et al., 1996) or inactivated by administration of NCAM anti-sera (Doyle et al., 1992). These agents produce deficits in hippocampal-dependent learning only when administered during the phase in which PSA-NCAM levels normally rise following initial training, termed consolidation (Doyle et al., 1992; Doyle and Regan, 1993; Welzl and Stork, 2003). Consistent with the theory that PSA-NCAM is crucial for memory acquisition and consolidation, mice treated with TMT prior to training on a spatial learning task demonstrated deficits 24–96 h after administration, measured by escape latency and percent of time spent in the platform quadrant. This relationship was demonstrated as a significant negative correlation between escape latency and PSA-NCAM expression at 96 h post TMT ($r = -0.54$, $p = 0.04$). The relationship between PSA-NCAM and spatial learning has been corroborated by studies which showed a more immediate and prolonged increase in PSA-NCAM in rats with superior water maze performance (Loscher et al., 2004). Because no differences in escape latency nor PSA-NCAM expression were observed between treatment groups at 168 h, it is expected that those animals that were impaired at 96 h would overcome the deficit and use alternative methods of finding the hidden platform (Pouzet et al., 2002). Therefore, following TMT exposure, hippocampal PSA-NCAM is reduced below baseline conditions, and furthermore, the learning-induced elevation in PSA-NCAM in the water maze is attenuated.

Since the treated mice and their vehicle controls performed equally well when the water maze platform was visible, the TMT-induced deficits in water maze acquisition and consolidation could not be attributed to alterations in the swimming ability or motivational state of the mice. In addition to an increase in escape latency, animals treated with TMT also spent less time in the quadrant in which the hidden platform was located ($p = 0.03$, data not shown). The impairment in spatial learning produced by TMT in the current studies is consistent with previous reports of spatial learning impairments, some of which accounted for motor impairments or motivational factors (Alessandri et al., 1994; Earley et al., 1992; Hagan et al., 1988; Walsh et al., 1982b). However, TMT administration also

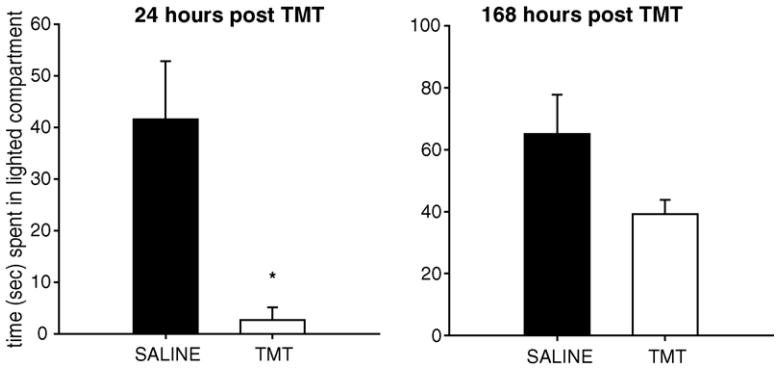

Fig. 5. Time spent in the light compartment in mice treated with TMT or saline and tested at either 24 or 168 h post-injection. $n = 8$ per group. (*) Indicates different from saline treated mice, $p < 0.05$.

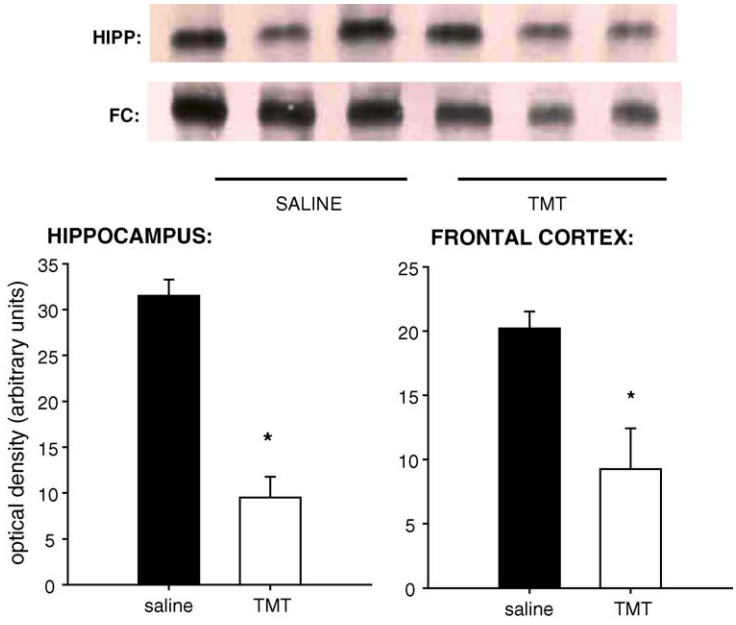

Fig. 6. PSA-NCAM levels in the hippocampus and frontal cortex taken from mice treated with TMT 2.25 mg/kg/i.p. or saline and sacrified 24 h after administration. $n = 3$ per group. (*) Indicates significantly different from saline treated mice using Fisher's PLSD, $p < 0.05$.

produced anxiogenic-like behaviors 96 h after its administration as evidenced by a decrease in time spent in a light compartment of a shuttlebox (data not shown). This increase in anxiety exhibited by mice at this time point following TMT administration may, in part, account for the amnesic effect.

A possible link between the anxiogenic and amnesic effects of TMT may be through the TMT-induced increase in plasma in corticosterone levels (Tsutsumi et al., 2002). However, while repeated restraint stress and chronic corticosterone administration have been shown to modulate PSA-NCAM expression in the dentate gyrus, acute stress does not change PSA-NCAM expression levels (Nacher et al., 2004; Pham et al., 2003). In addition, the effects of stress and/or elevated corticosteroids on memory consolidation in the water maze are complex. Moderate activation of the stress response facilitates memory formation while a high level of activation impairs this process

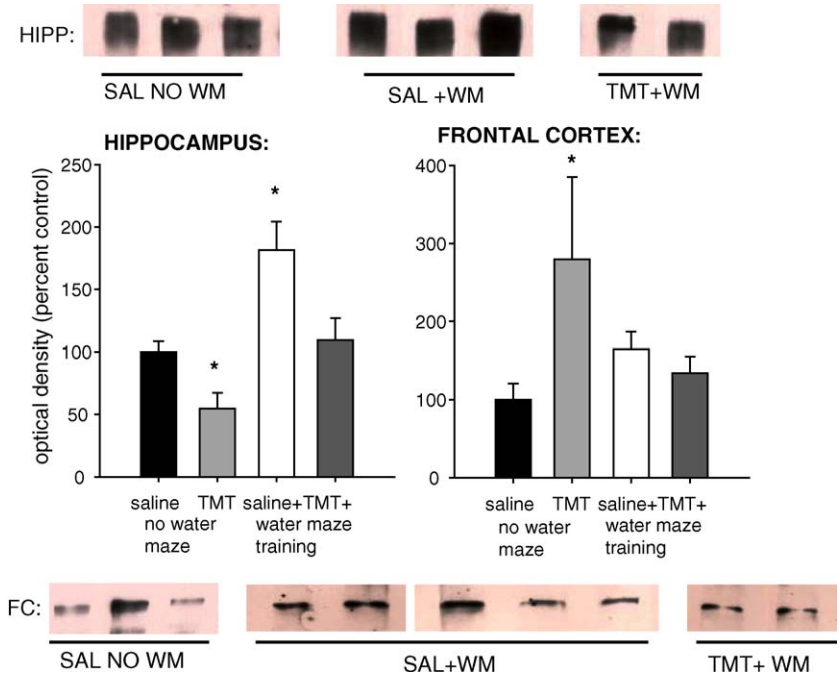

Fig. 7. PSA-NCAM levels in the hippocampus and frontal cortex following TMT and saline treatment. Mice were sacrificed 108 h after injection (12 h after water maze training, which occurred 96 h after treatment). $n = 4$ saline no WM, $n = 10$ each saline + WM and TMT + WM. PSA-NCAM levels from mice treated with saline or TMT without water maze training were determined in a separate study, $n = 4$ saline and $n = 5$ TMT treatment. (*) Indicates significantly different from saline treated mice using Fisher's PLSD, $p < 0.05$.

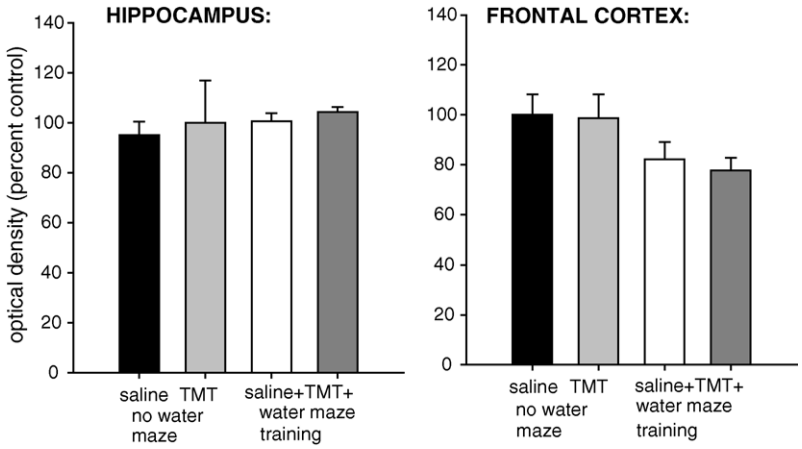

Fig. 8. NCAM-180 expression in the hippocampus (left) and frontal cortex (right) in animals sacrificed 108 h after TMT exposure (12 h post training). There were no significant effects of treatment or water maze training ($p > 0.05$ in both brain regions).

(Sandi et al., 1997; Akirav et al., 2004; Szuran et al., 2000; Zaharia et al., 1996; Yau et al., 1995). Therefore, the role of acutely elevated corticosterone on TMT-induced spatial memory deficits and changes in PSA-NCAM expression deserves further investigation.

The elevation in PSA-NCAM in the cortex at 96 h post-treatment may serve as a compensatory or reparative response following injury to the hippocampus. Recent studies in other mouse strains have demonstrated a concomitant loss of neuron number with increased neurogenesis following TMT treatment in younger mice (Harry et al., 2004). While previous studies have not reported widespread neuron death using a similar dose in BALB/c mice (Dey et al., 1997), it is likely that TMT produced terminal swelling, the repair of which would be consistent with an increase in PSA-NCAM. This theory is also consistent with previous experiments which reported an increase in PSA-NCAM in the dentate gyrus following ischemia (Iwai et al., 2001) or the contralateral ganglia following ciliary nerve crush (DeStephano et al., 2001).

At 168 h post-treatment, both saline and TMT-treated mice learned the water maze task effectively and exhibited an elevation in PSA-NCAM that approached significance. Therefore, the effects of TMT on spatial learning at earlier time points are unlikely to be related to neuropathology of the limbic system, as these deficits resolved at 1 week and neuronal necrosis or granule cell death would not be expected at doses below 3.0 mg/kg in BALB/c mice (Chang et al., 1983; Dey et al., 1997; Wenger et al., 1984a). Likewise, doses of TMT (3.0 mg/kg) which did produce death of granule cells in the dentate gyrus also resulted in hyperactivity which peaked 5 days after treatment (Wenger et al., 1984a). In contrast, TMT at 2.25 mg/kg in the current study produced a reduction in locomotor activity which was most apparent at 24 h post-treatment and disappeared by 168 h. Impairments in motor

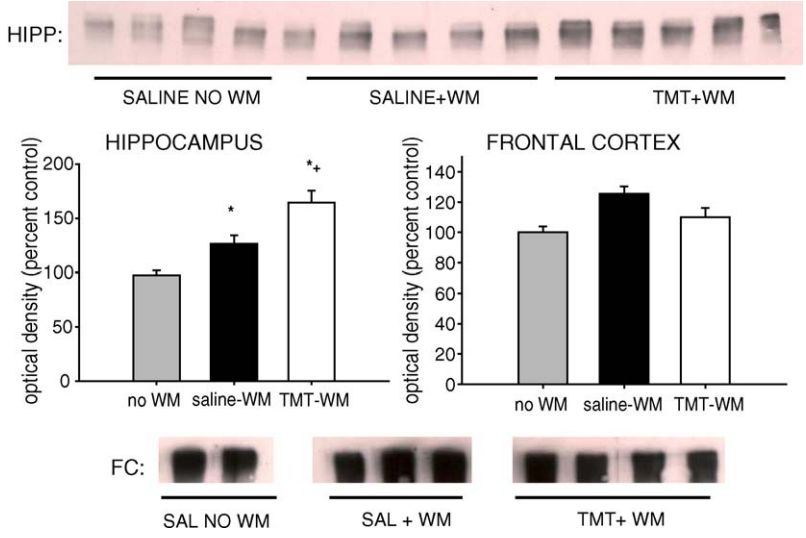

Fig. 9. Post-water maze training exposure to TMT-induced changes in PSA-NCAM levels in the hippocampus and frontal cortex. Mice were treated with TMT 12 h after three consecutive days of water maze training, and tested 96 h after TMT administration. $n = 4$ saline + no WM, $n = 6$ saline + WM and $n = 8$ TMT + WM. (*) Indicates significantly different from saline–no WM treated mice (control exposed) and (+) indicates significantly different from saline + WM treated mice using Fisher's PLSD, $p < 0.05$.

activity, light avoidance and acquisition of spatial learning resolved in TMT-treated mice at the same time point that PSA-NCAM levels returned to normal (168 h). This is consistent with the observed rescue of some behavioral deficits in the NCAM knockout mouse following transgenic re-expression of NCAM-180 (Stork et al., 2000).

4.2. Reconsolidation

Mice treated with TMT following 3 days of maze training (during a period of reconsolidation) showed a mild impairment in escape latency 96 h post-TMT, however, escape latency returned to pretreatment values within a few trials. This effect is similar to the transient amnesic effects of electroshock or protein synthesis inhibitors during periods of reconsolidation in other paradigms (Misanin et al., 1968; Nader et al., 2000; Sara, 2000). Past studies have linked TMT-induced deficits in memory recall with morphological damage in the dentate gyrus (Walsh et al., 1982b), therefore dysregulation in PSA-NCAM expression following TMT may not be sufficient to produce long-term amnesia. Because both groups showed a comparable reduction in escape latency on the testing day, the increase in PSA-NCAM levels following sacrifice at 12 h post-training was consistent with the other results.

The focus of the current studies was to investigate the TMT-induced disruption of PSA-NCAM expression during acquisition, consolidation and reconsolidation of a spatial learning task, and a transfer test was not performed. However, it is expected that those animals that were impaired during training would not learn the task effectively and would perform poorly on recall (Sandi et al., 2004). These results demonstrate that the spatial learning impairment produced by TMT exposure reported by others is dependent on the period of learning during which it is administered. TMT-induced perturbation of PSA-NCAM levels was detected in both the hippocampus and cortex at 24–96 h post-treatment, therefore future studies will assess the effects of neurotoxicant exposure on PSA-NCAM expression at other periods of task learning which further target these neuroanatomical substrates.

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